Use of Superficial Markers for Estimation of the Intensity of Immunological Process in Multiple Sclerosis

G. V. Poryadin, A. I. Makarkov, A. N. Boiko, Zh. M. Salmasi, T. L. Demina, and E. I. Gusev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 9, pp. 342-345, September, 1997
Original article submitted November 22, 1996

The surface phenotype of peripheral blood lymphocytes was studied in patients with different stages of multiple sclerosis and in healthy donors. There was no differences in the $CD3^+$, $CD5^+$, and $CD8^+$ lymphocyte counts, while the count of $CD4^+$ lymphocytes was slightly higher in patients with clinically active disease, i.e., with pronounced neurological symptoms. At the same time, the $CD25^+$ lymphocyte count was considerably higher predominantly in patients with severe multiple sclerosis. It is suggested that the count of $CD25^+$ lymphocytes can be used to determine the stage of multiple sclerosis.

Key Words: *multiple sclerosis; lymphocytes; surface phenotype*

The hybridoma technology has provides a considerable body of evidence on the surface phenotype of lymphocytes in various autoimmune diseases, including multiple sclerosis (MS). So far, these data have no clinical application, since these is controversy over the relationships between changes in the number of cells with certain phenotype and clinical manifestations of a disease. Activation of pathological process in MS may occur asymptomatically [10], and its timely diagnosis allows for the correct choice of adequate therapy.

In the 1970s-1980s, it was demonstrated that the count of $CD8^+$ lymphocytes and the CD4/CD8 ratio (immunoregulatory index) increase in exacerbated MS, which was interpreted as insufficient immunosuppression [3-5]. However, further investigations with the use of more sensitive methods did not confirm this relationship. Shifts in the lymphocyte count proved to be associated with complex processes and presumably reflect not the intensity of pathological processes but rather heterogeneity of the CD8 lymphocyte population, discrepancy between phenotype and functional activity of cells, and circadian fluctuations in the T-cell population [13,14]. Different approaches to the estimation of MS severity, use of different clinical scales, subclinical and pseudoexacerbations, secondary infectious diseases, and other MS-related factors which are not always manifested in the course of the disease contribute to variations in the lymphocyte count [8]. It was reported that changes in the count of $CD8^+$ cells in peripheral blood and cerebrospinal fluid occur in some patients with active MS and in 50-70% patients with exacerbated MS [12]. It should be noted that fluctuations in the $CD8^+$ lymphocyte count were also observed in healthy relatives of MS patients, which points to a possible genetically determined proneness to MS [9]. Thus, the attempts to use lymphocyte phenotyping in clinical immunology have been met with scepticism. It was stated that changes in the counts of regulatory cells are difficult to interpret and have no diagnostic validity [6]. On the other hand, activation of the immune system in MS is beyond any doubt [4]. The aforesaid prompted us to find out the most informative parameters for the diagnosis of active immunopathological process in MS.

Russian State Medical University, Moscow

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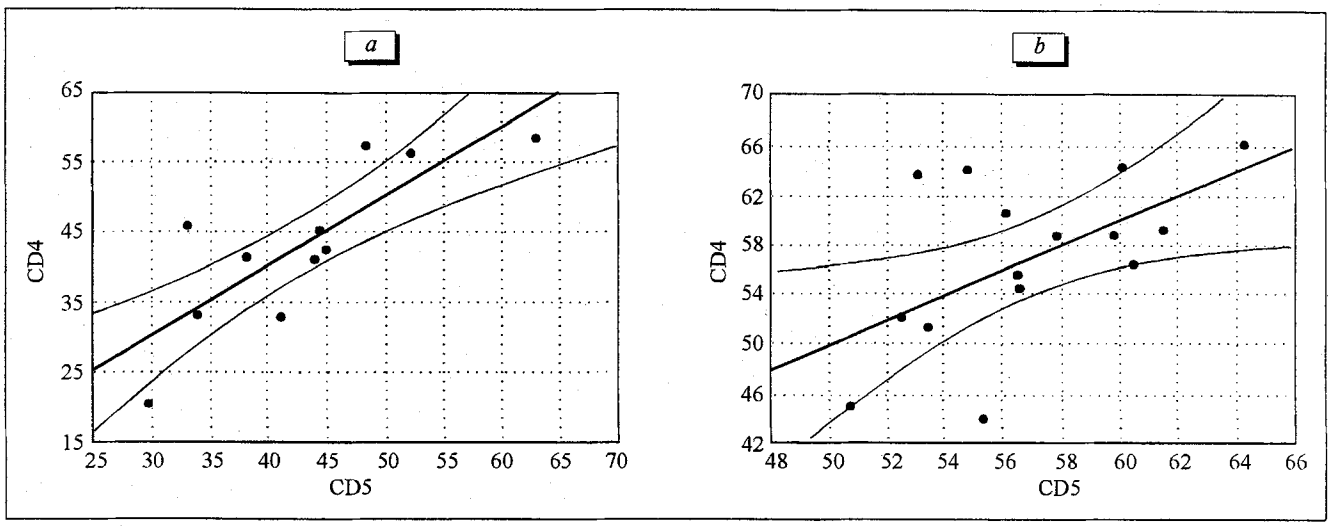

Fig. 1. Relationship between the counts of CD4⁺ and CD5⁺ lymphocytes in patients with stable MS (*a*, r=0.73) and exacerbated MS (*b*, r=0.51).

In the present study we investigated T-cell markers in patients with different stages of MS. We compared the counts of lymphocytes expressing CD3 or CD5, differential antigens CD4 and CD8, and the activation marker CD25 (receptor for interleukin-2).

MATERIALS AND METHODS

Lymphocytes were obtained from 31 healthy subject and 56 MS patients. According to the activity of pathological process, the patients were divided into two groups: 31 patient with MS remaining stable within the last 2 months (group 1), 25 patients with increasing intensity of neurological symptoms (group 2). According to the severity of MS, they were also divided into two groups: group 1 included 34 patients with mild and moderate MS (Kurtske index equal to 1-4) and group 2 included 22 patients with sever MS (Kurtske index was 5 and higher) [11].

Lymphocytes were isolated from peripheral blood by centrifugation on a Ficoll-Verografin gradient and phenotyped by indirect immunofluorescence with murine monoclonal antibodies and FITC-conjugated Fab fragments of goat anti-mouse immunoglobulins. The preparations were viewed in a LYuMAM-I3 luminescence microscope. At least 200 cells were analyzed [7]. Cells morphologically similar to monocytes were excluded. In each experiment lymphocytes were tested for viability (98% in the trypan blue exclusion test) and nonspecific reaction with labeled serum (<4%).

The clinical status of a patients was estimated before immunological studies, and immunologists had no information about the patient. The results were statistically analyzed using Student's t test and Spearman correlation coefficient.

RESULTS

The counts of CD3⁺ and CD5⁺ lymphocytes in MS patients were practically the same as in healthy donors (Table 1). A slight increase in the CD4⁺ lymphocyte count (p<0.05) was observed in patients with increasing intensity of neurological symptoms, which is in agrees with our previous observations [1,2]. The were no changes in the count of CD8⁺ lymphocytes. We did not find any statistically significant phenotypic differences in peripheral blood lymphocytes

TABLE 1. Phenotyping of Lymphocytes from Peripheral Blood of Patients with MS of Various Severity

Marker	Donors	All MS patients	Stable MS	Exacerbated MS
CD3	68.58±1.36	66.89±1.65	67.42±2.07	66.43±2.56
CD5	55.18±1.46	55.59±1.73	56.41±2.97	54.96±2.11
CD4	44.24±0.69	46.92±1.25	45.69±1.98	47.94±1.59*
CD8	25.91±1.18	26.37±1.39	27.80±2.16	25.22±1.81
CD25	11.60±2.27	16.92±1.11*	11.33±0.99	21.42±1.39**
IRI	1.79±0.09	2.11±0.14	1.96±0.20	2.23±0.19

Note. *p<0.05 compared with donors; **p<0.001 compared with patients with stable MS. *IRI*) immunoregulatory index.

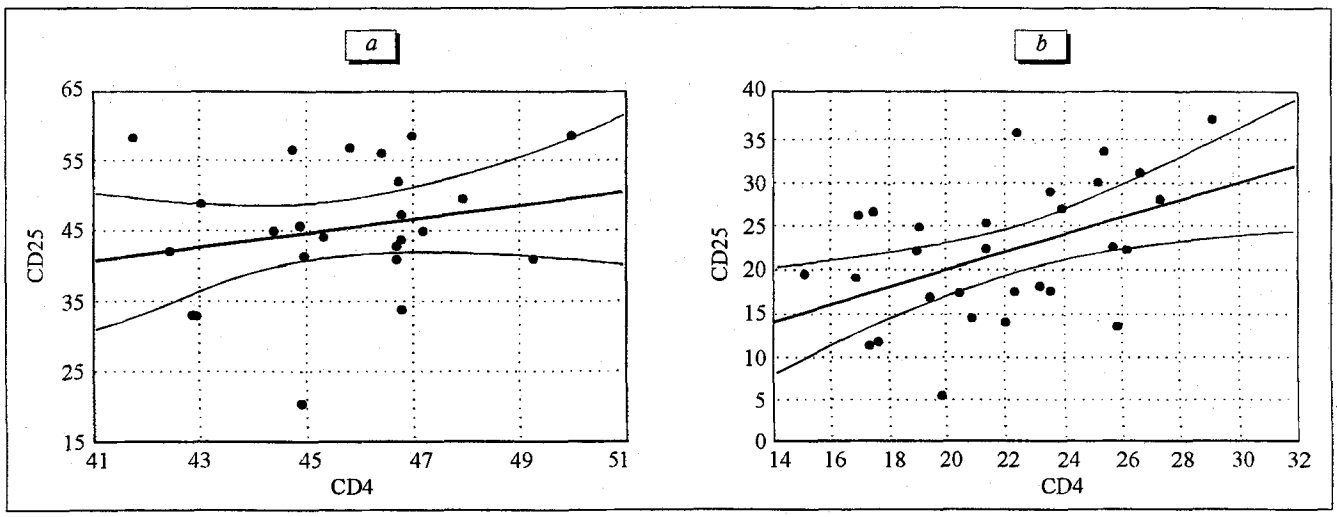

Fig. 2. Relationship between the counts of $CD4^+$ and $CD5^+$ lymphocytes in patients with stable MS (*a*, r=0.21) and exacerbated MS (*b*, r=0.45).

from MS patients with different severity of neurological symptoms (data not shown).

The count of lymphocytes expressing the early activation marker CD25 proved to be most informative. The count of $CD25^+$ cells was higher in MS patients than in healthy subjects. It is noteworthy that the count of $CD25^+$ lymphocytes increased only in patients in which the severity of neurological disorders steadily increased during a 2-month period before the investigation. In MS patients this parameter increased 2-fold in comparison with that in healthy subjects ($p<0.001$). By contrast, in patients with stable MS the mean count of $CD25^+$ cells did not differ from that in healthy subjects. There was no correlation between the count of $CD25^+$ lymphocytes and severity of neurological symptoms.

In patients with stable MS, the count of $CD4^+$ lymphocytes correlated with that of $CD5^+$ (but not $CD3^+$) lymphocytes (r=0.73, $p<0.01$, Fig. 1). This can be interpreted as an indirect evidence that CD4 and CD5 antigens are expressed on the surface of the same cells or the expression of CD5 antigen on $CD8^+$ lymphocytes is so low that these cells cannot be visualized by the immunofluorescence method. There was no statistically significant correlation between the counts of $CD4^+$ and $CD5^+$ lymphocytes in patients with increasing intensity of neurological symptoms; however, the count of $CD4^+$ cells correlated with that of $CD25^+$ cells (r=0.45, $p<0.05$, Fig. 2). This information is useless for the understanding of the role of membrane antigens in the activation events. Previously, we reported that $CD4^+$ lymphocytes and increased binding of interleukin-2 may have a role in the activation of immunological process in MS [2,10]. The results of the present study also point to the special role of lymphocytes carrying CD4 antigen or the interleukin-2 receptor (CD25) in MS exacerbation.

Thus, the $CD25^+$ lymphocyte count can be useful for assessing the severity of immunological disorders in MS and probably for differential diagnosis between MS an noninflammatory and/or autoimmune neurological diseases. We observed an increase in the $CD25^+$ lymphocyte count in other autoimmune diseases (systemic lupus erythematosus) and viral infections (for example, in exacerbated herpetic infection).

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